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Synthesis, Spectral Characterization and Screening of New Triazolo Thienopyrimidine Derivatives for their Antimicrobial Activity

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ABSTRACT

In this study a series of novel 2-Methyl-3-(1-methyl-1H-[1,2,3] triazol-4-yl-methyl)-5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-d]pyrimidine-4-one derivatives (IVa-e) were synthesized by treating 2-Methyl-3-prop-2-ynyl-5,6,7,8-tetrahydro-3H-benzo[4,5]thieno[2,3-d] pyrimidine-4-one (III) and its derivatives with alkyl azides in the presence of copper sulphate and dimethylsulphoxide. By treating 2-Methyl-5,6,7,8,-tetrahydrobenzo[b]thieno[2,3-d]pyrimidine-4-one(II) and its derivatives with propargyl bromide in the presence of potassium carbonate in acetone results in the formation of N-substituted compound-III and its derivatives. The compound-II and its derivatives were synthesized by treating 2-amino-thiophene -3-carboxylic acid ethyl ester (I) with alkyl/aryl nitrile in the presence of dry HCl and 1,4-dioxane. The newly synthesized compounds (IVa-h) were purified, separated, and characterized by TLC, IR, [1]HNMR and Mass spectra. These representative analogues were screened for invitro antimicrobial activity. The compounds exhibited significant antibacterial and antifungal activities.

Key words : *Thieno pyrimidines, dimethylsulphoxide, Antimicrobial IC50 values.*

INTRODUCTION

Pyrimidine derivatives are very important heterocyclic compounds especially in life sciences, medicinal chemistry and in pesticidal chemistry and continue to attract great interest due to a wide variety of interesting biological activities such as anti-inflammatory, anticancer, antiviral, and antimicrobial activities. Fused pyrimidinones were reported to exhibit wide spectrum of activities like bronchodilatory [1], antihistaminic [2], anticancer [3] among the pyrimidinones, thienopyrimidines have been evaluated pharmacologically and used as antimicrobial [4], analgesic [5], anti-inflammatory [5] and antiviral [6] agents.

1,2,4-triazoles possess important pharmacological activities like anti-inflammatory [7], antifungal [8] and cytotoxic [9] activities. Fused triazoles are proved to have diverse applications as antibacterial [10] and anticancer [11] agents. Recent literature reveals that when one heterocyclic system is coupled with another, a molecule with enhanced biological activity is produced (Click Chemistry).

Click chemistry is a modular approach that uses only the most practical and reliable chemical transformations. Which describes the chemistry tailored to generate substances quickly and reliably by joining small units together[12]. Its applications are increasingly found in all aspects of drug discovery, ranging from lead finding through combinatorial chemistry and target template *in situ* chemistry, to proteomics and DNA research, using bio-conjugation reactions.

Considering all these potential biological activities of thienopyrimidines and triazoles which were reported to be more potent and less toxic, it has been felt worthwhile to take up the present investigation in an effort to incorporate triazole ring system into thienopyrimidine derivatives with methylene bridge to synthesize novel N-alkynyl triazolo thienopyrimidines as antimicrobial agents.

MATERIALS AND METHODS

All the chemicals used were Aldrich, Fluka and Merck company. Thin layer chromatography (TLC) was performed on E.Merk AL silica gel 60 F254 plates and visualized under UV light. The IR spectra were determined in a Perkin-Elmer transform (FTIR spectrum). ^{1}H NMR spectra were recorded on varian EM-360(400MHz mercury plus) spectrometer in DMSO or $CDCl_3$ and calibrated using solvent signals. All chemical shifts recorded in δ (ppm) using TMS as an internal standard. The mass spectra were recorded on Agilent ion trap MS at energy of ionizing electron equal to 70ev.

2-Methyl-5,6,7,8,-tetrahydrobenzo[b]thieno[2,3-d]pyrimidine-4-one(II) was prepared from 2-amino-thiophene -3-carboxylic acid ethyl ester (I) with alkyl/aryl nitrile in the presence of dry HCl and 1,4-dioxane. The synthesized compound-II was treated with propargyl bromide in the presence of potassium carbonate in acetone results in the formation of N-substituted compound-III and its derivatives. The synthesized compound-III and its derivatives were treated with alkyl azides in the presence of copper sulphate and dimethylsulphoxide yields 2-Methyl-3-(1-methyl-1H-[1,2,3]triazol-4-yl-methyl)-5,6,7,8-tetrahydra-benzo[4,5] thieno[2,3-d]pyrimidin-4-one (IV) and its derivatives. The newly synthesized triazolo thienopyrimidine derivatives were characterized by both physical and spectral data. These compounds were evaluated for their antimicrobial activity. The melting points of synthesized compounds were determined with an electro thermal melting point apparatus.

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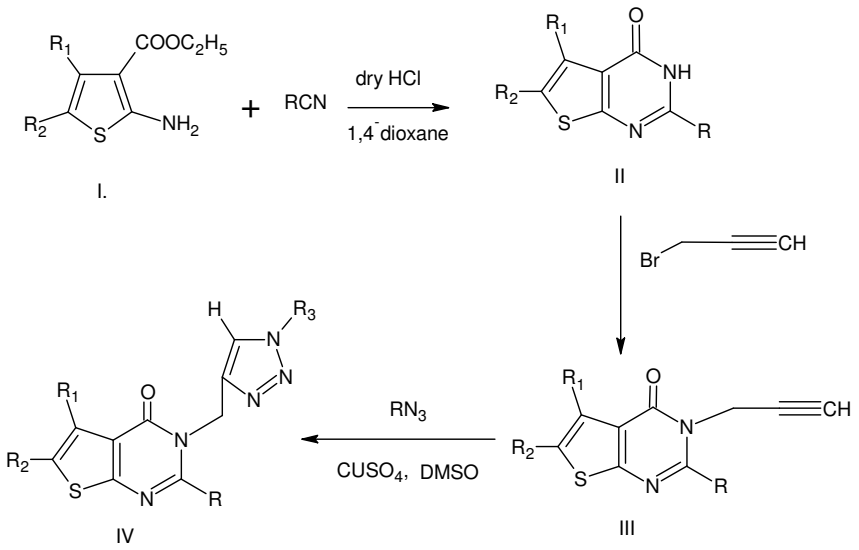
Scheme:



R = CH_3, C_6H_5, R_1 and R_2 = $-(CH_2)_4$, $-(CH_2)_3$, R_3 = H, CH_3, C_2H_5, C_3H_7.

Synthesis of Triazolo Thienopyrimidines:

Synthesis of Ethyl 2-Amino- 4,5- Substituted Thiophene- 3-Carboxylate (I):

An equimolar quantities of (0.01 moles) of sulphur, ethyl cyano acetate and respective ketone were taken in 15 ml of ethanol in conical flask. The mixture was stirred for 5 min and morpholine was added slowly at 50°c and stirred continuously for 5hours at room temperature and the complete mixture solution was kept in refrigerator overnight. The crystals obtained were collected by filtration and recrystallized with ethanol.

Table No. 1:

S. No.	Compound	R_1	R_2	Molecular formula	Melting point (°C)	% yield
1	Ia	$-(CH_2)_4-$		$C_{11}H_{15}NO_2S$	110-115	85
2	Ib	CH_3	CH_3	$C_9H_{13}NO_2S$	100-105	65
3	Ic	$-(CH_2)_3-$		$C_{10}H_{13}NO_2S$	95-98	50

Step-1: Synthesis of 2,5,6-Substituted Thienopyrimidin-4-One (II):

2-Amino thiophen-4,5-substituted-3-carboxylic acid ethyl ester (0.001moles) was treated with alkyl or aryl nitrile (0.002 moles) in dry dioxane. Dry HCl gas was passed using dip rod till the starting material was disappeared in TLC. Excess nitrile present in the reaction mixture was removed under pressure and the solution was poured in crushed ice and the mixture was neutralized with sodium bicarbonate solution. The precipitate was collected by vaccum filtration.

Table No. 2:

S. No.	Compound	R_1	R_2	R	Molecular formula	Melting point (°C)	% yield
1	IIa	$-(CH_2)_4-$		CH_3	$C_{11}H_{12}N_2OS$	250-256	80
2	IIb	$-(CH_2)_4-$		C_6H_5	$C_{16}H_{14}N_2OS$	275-280	70
3	IIc	CH_3	CH_3	CH_3	$C_9H_{10}N_2OS$	270-275	80
4	IId	$-(CH_2)_3-$		CH_3	$C_{10}H_{10}N_2OS$	280-285	75

Step-2: Synthesis of Propargylated Thienopyrimidines (III):

Compound-II (3 mmoles) and potassium carbonate (6 mmoles) in 1:2 ratio were taken in 20 ml of acetone in two necked flask. To this mixture propargyl bromide was added dropwise under heating and refluxed for 5 hours, and the mixture was concentrated in vacuo and added to the ice cold water. The obtained N-propargylated product was collected by filtration and separated by column chromatography.

Table No. 3:

S. No.	Compound	R	R_1	R_2	Molecular formula	Melting point (°C)	% yield
1	IIIa	CH_3	$-(CH_2)_4-$		$C_{14}H_{14}N_2OS$	175-180	10.41
2	IIIb	C_6H_5	$-(CH_2)_4-$		$C_{19}H_{16}N_2OS$	175-180	20.83
3	IIIc	CH_3	CH_3	CH_3	$C_{12}H_{12}N_2OS$	165-170	28.73
4	IIId	CH_3	$-(CH_2)_3-$		$C_{13}H_{12}N_2OS$	180-185	21.06

Step-3: Synthesis of 1,4-Substituted 1,2,3- Triazolyl Thienopyrimidines (IV):

Equimolar proportions of N-substituted alkynyl thienopyrimidine and copper iodide were taken in DMSO in round bottomed flask and cooled to 0°c. To this an alkyl azide was added dropwise under stirring, and the stirring was continued for 12 hours. The concentrated reaction mixture was poured in to the crushed ice. The crude triazolyl thienopyrimidine product was collected by extraction with ethyl acetate. The product was purified by column chromatography.

Table No. 4 :

S. No.	Compound	R	R_1	R_2	R_3	Molecular formula	Mol.wt	Melting point (°C)	% yield
1	IVa	CH_3	$-(CH_2)_4-$		CH_3	$C_{15}H_{17}N_5OS$	315	198-205	86.06
2	IVb	CH_3	$-(CH_2)_4-$		C_2H_5	$C_{16}H_{19}N_5OS$	329	222-227	70.58
3	IVc	CH_3	$-(CH_2)_4-$		C_3H_7	$C_{17}H_{21}N_5OS$	343	200-205	69.81

4	**IVd**	CH_3	CH_3	CH_3	CH_3	$C_{13}H_{15}N_5OS$	289	200-208	67.46
5	**IVe**	CH_3	CH_3	CH_3	C_2H_5	$C_{14}H_{17}N_5OS$	303	190-196	58.12
6	**IVf**	CH_3	CH_3	CH_3	C_3H_7	$C_{15}H_{19}N_5OS$	317	195-200	54.94
7	**IVg**	CH_3	$-(CH_2)_3-$		CH_3	$C_{14}H_{15}N_5OS$	301	225-228	60.70
8	**IVh**	CH_3	$-(CH_2)_3-$		C_2H_5	$C_{15}H_{17}N_5OS$	315	220-225	57.92

Compound(IVa):

2-Methyl-3-(1-methyl-1H-[1,2,3]triazol-4-ylmethyl)-5,6,7,8-tetrahydra-benzo[4,5]thieno[2,3-d]pyrimidine-4-one:

FT-IR in cm^{-1}(KBr) 3445(νN-H), 3073(C-H Strech in alkenes), 2926(C-H Strech in methylene), 2855(C-H Strech in methyl), 1672(νC=O), 1551(C=N), 790.34(νC-S)., **^{1}H NMR** ($CDCl_3$, δppm):0.8(s, 3H,CH_3), 1.3(s, 4H, $2CH_2$), 2.4- 2.9(m, 4H, $2CH_2$), 3.7(s, 3H, N-CH_3), 4.3(s, 2H, N-CH_2) and 7.3(s, 1H, C-H)., **ESI-MS** m/z: 315(M+H)$^+$m/z calculated for $C_{15}H_{17}N_5OS$ [M+H]$^+$ 314 found 314.18.

Compound(IVb):

2-Methyl-3-(1-ethyl-1H-[1,2,3]triazol-4-ylmethyl)-5,6,7,8-tetrahydra-benzo[4,5]thieno[2,3-d]pyrimidine-4-one:

FT-IR in cm^{-1}(KBr) 3445(ν N-H), 3073(C-H Strech in alkenes), 2926(C-H Strech in methylene), 2855(C-H Strech in methyl), 1672(ν C=O), 1551(C=N), 790.34(νC-S)., **^{1}H NMR** ($CDCl_3$, δppm):0.8(s, 3H,CH_3), 1.3(s, 3H, CH_3), 1.7- 2.0(d, 4H, $2CH_2$), 2.5-2.8(d, 4H, $2CH_2$) 3.9(s, 2H, N-CH_2), 4.3(s, 2H, N-CH_2) and 7.3(s, 1H, C-H)., **ESI-MS** m/z: 329(M+H)$^+$m/z calculated for $C_{16}H_{19}N_5OS$ [M+H]$^+$ 328 found 328.28.

Compound(IVc):

2-Methyl-3-(1-Propyl-1H-[1,2,3]triazol-4-ylmethyl)-5,6,7,8-tetrahydra-benzo[4,5]thieno[2,3-d]pyrimidine-4-one:

FT-IR in cm^{-1}(KBr) 3445(ν N-H), 3073(C-H Strech in alkenes), 2926(C-H Strech in methylene), 2855(C-H Strech in methyl), 1672(ν C=O), 1551(C=N), 790.34(ν C-S)., **^{1}H NMR** ($CDCl_3$, δppm):0.8(s, 3H,CH_3), 1.3(s, 3H, CH_3), 1.7- 2.0(d, 4H, $2CH_2$), 2.1-2.3(d, 4H, $2CH_2$) 2.5-2.8(d, 4H, $2CH_2$) 3.9(s, 2H, N-CH_2), 4.3(s, 2H, N-CH_2) and 7.3(s, 1H, C-H)., **ESI-MS** m/z: 343(M+H)$^+$m/z calculated for $C_{17}H_{21}N_5OS$ [M+H]$^+$ 342 found 342.73.

Compound(IVd):

2,5,6-Trimethyl-3-(1-methyl-1H-[1,2,3]triazol-4-ylmethyl)-3H-thieno[2,3-d]pyrimidine-4-one:

FT-IR in cm^{-1}(KBr) 3445(ν N-H), 3073(C-H Strech in alkenes), 2926(C-H Strech in methylene), 2855(C-H Strech in methyl), 1672(ν C=O), 1551(C=N), 790.34(ν C-S).,**^{1}H NMR** ($CDCl_3$, δppm):0.9(s, 3H,CH_3), 1.4(d, 3H, CH_3), 2.3- 2.7(m, 4H, $2CH_2$), 3.7(s, 3H, N-CH_2), 4.3(s, 2H, N-CH_2) and 7.1(s, 1H, C-H), **ESI-MS** m/z: 289(M+H)$^+$m/z calculated for $C_{13}H_{15}N_5OS$ [M+H]$^+$ 288 found 288.15.

Compound(IVe):

2,5,6-Trimethyl-3-(1-ethyl-1H-[1,2,3]triazol-4-ylmethyl)-3H-thieno[2,3-d]pyrimidine-4-one:

FT-IR in cm^{-1}(KBr) 3445(ν N-H), 3073(C-H Strech in alkenes), 2926(C-H Strech in methylene), 2855(C-H Strech in methyl), 1672(ν C=O), 1551(C=N), 790.34(ν C-S).,**^{1}H NMR** ($CDCl_3$, δppm):0.9(s, 3H,CH_3), 1.4(d, 3H, CH_3), 2.3- 2.7(m, 4H, $2CH_2$), 3.7(s, 2H, N-CH_2), 4.2(s, 2H, N-CH_2) and 7.3(s, 1H, C-H)., **ESI-MS** m/z: 303(M+H)$^+$m/z calculated for $C_{14}H_{17}N_5OS$ [M+H]$^+$ 302 found 302.80.

Compound(IVf):

2,5,6-Trimethyl-3-(1-propyl-1H-[1,2,3]triazol-4-ylmethyl)-3H-thieno[2,3-d]pyrimidine-4-one:

FT-IR in cm^{-1}(KBr) 3445(ν N-H), 3073(C-H Strech in alkenes), 2926(C-H Strech in methylene), 2855(C-H Strech in methyl), 1672(ν C=O), 1551(C=N), 790.34(νC-S)., **^{1}H NMR** ($CDCl_3$, δppm):0.9(s, 3H,CH_3), 1.4(d, 3H, CH_3), 2.5- 2.9(m, 6H, $3CH_2$), 3.7(s, 2H, N-CH_2), 4.2(s, 2H, N-CH_2) and 7.3(s, 1H, C-H)., **ESI-MS** m/z: 317(M+H)$^+$m/z calculated for $C_{15}H_{19}N_5OS$ [M+H]$^+$ 316 found 316.9.

Compound(IVg):

2-methyl-3-(1-methyl-1H-[1,2,3]triazol-4-ylmethyl)-1,2,3,5tetrahydro-cyclopenta[4,5]thieno[2,3-d]pyrimidine-4-one:

FT-IR in cm^{-1}(KBr) 3445(ν N-H), 3073(C-H Strech in alkenes), 2926(C-H Strech in methylene), 2855(C-H Strech in methyl), 1672(ν C=O), 1551(C=N), 790.34(ν C-S)., **^{1}H NMR** ($CDCl_3$, δppm):0.9(s, 3H,CH_3), 1.3(s, 2H, CH_2), 2.5(s, 3H, CH_3), 2.8(s, 3H, CH_2) 2.9-3.2(d, 2H, CH_2), 3.5(s, 3H, N-CH_3), and 7.3(s, 1H, C-H)., **ESI-MS** m/z: 301(M+H)$^+$m/z calculated for $C_{14}H_{15}N_5OS$ [M+H]$^+$ 300 found 300.64.

Compound(IVh):

2-methyl-3-(1-ethyl-1H-[1,2,3]triazol-4-ylmethyl)-1,2,3,5tetrahydro-cyclopenta[4,5]thieno[2,3-d]pyrimidine-4-one:

FT-IR in cm^{-1}(KBr) 3445(ν N-H), 3073(C-H Strech in alkenes), 2926(C-H Strech in methylene), 2855(C-H Strech in methyl), 1672(ν C=O), 1551(C=N), 790.34(ν C-S)., **^{1}H NMR** ($CDCl_3$, δppm):0.9(s, 3H,CH_3), 1.3(s, 2H, CH_2), 2.5(s, 3H, CH_3), 2.8(s, 3H, CH_2) 2.9-3.2(d, 2H, CH_2), 3.7(s, 2H, N-CH_2), 6.2(s, 2H, N-CH_2) and 7.3(s, 1H, C-H)., **ESI-MS** m/z: 315(M+H)$^+$m/z calculated for $C_{15}H_{17}N_5OS$ [M+H]$^+$ 314 found 314.98.

Antimicrobial Activity:

All the newly synthesized compounds were screened for their antibacterial and antifungal activities. For antibacterial study microorganisms employed were E-coli, K.pneumonia (gram –ve bacteria), S.aureus, B.subtilis (gram +ve bacteria). For antifungal study microorganism employed was Candida albicans. Both antibacterial and antifungal activities were investigated by using filter paper strip method in nutrient agar medium, and Sabouraud dextrose of czapexs dox agar medium by using drug standards Streptomycin and Fluconazole. The compounds were tested invitro for their antibacterial activity which are pathogenic to human beings. The Inoculation period for the test organisms in nutrient agar media was found to be 37± 1ºC for 24hrs, whereas for antifungal activity the zone of inhibition in mm was measured after 24 hrs of inhibition at 25^{0}C.

RESULTS AND DISCUSSIONS

Formation of 1,4-substituted-1,2,3-triazolyl thieno pyrimidines **(IV a-h)** were confirmed by recording their IR, ^{1}HNMR and Mass spectral analysis. **IR** spectrum of compound IVa showed absorption bands at 3445, 3073, 2926, 2855, 1672, 1551, and 790.34 cm^{-1} due to ν N-H, C-H str in alkene, C-H stretching in methylene group, C-H str in methyl, C=O, C=N and C-S respectively. Similarly the mass spectrum was recorded and reported as (M+1) values. For the compound IVa. a molecular weight 315 is consistence with the molecular formula $C_{15}H_{17}N_5OS$. The values for the remaining compounds have been presented under the experimental part.

From the antimicrobial study the results conclude that among the newly synthesized triazolyl thienopyrimidines, IVa, IVc, and IVe exhibited good antibacterial activity against K.pneumoniae, E-coli, S,aureus, B.subtilis, and the compound IVa and IVh exhibited better antifungal activity against Candida albicans.

Table No. 5:

S.NO	R	R_1	R_2	R_3	Antibacterial potency(IC_{50})				Antifungal(IC_{50})
					E.c	K.p	S.a	B.s	C.a
Via	CH_3	$-(CH_2)_4-$		CH_3	384.6	360.0	330.2	374.8	285
VIb	CH_3	$-(CH_2)_4-$		C_2H_5	454.6	505.7	399.4	384.6	750
VIc	CH_3	$-(CH_2)_4-$		C_3H_7	518.3	296.1	415.9	527.0	435
VId	CH_3	CH_3	CH_3	CH_3	312.3	366.2	412.5	355	355
VIe	CH_3	CH_3	CH_3	C_2H_5	306.5	296.1	410.2	360	360
VIf	CH_3	CH_3	CH_3	C_3H_7	306.5	390.1	434.0	520	520
VIg	CH_3	$-(CH_2)_3-$		CH_3	485.4	501.6	415.3	535.9	435
VIh	CH_3	$-(CH_2)_3-$		C_2H_5	553.1	455.0	335.1	447.2	280

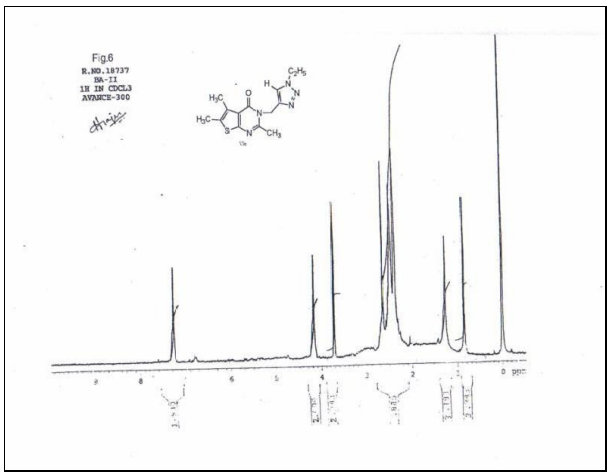


Fig. 1: ^{1}H NMR

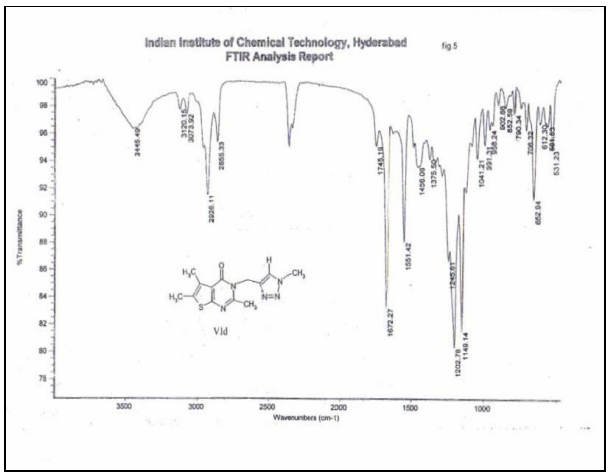


Fig. 2: FT-IR

CONCLUSION

In conclusion the Synthesis, Characterization, Pharmacological analysis of 2-Methyl-3-(1-methyl-1H-[1,2,3]triazol-4-ylmethyl)-5,6,7,8-tetrahydra-benzo[4,5]thieno[2,3-d]pyrimidine-4-one and its analogues was done. The representative examples were Screened in vitro antibacterial activity and antifungal activity. The biological evaluation indicate that the molecules IVa, IVc, and IVe exhibited better antibacterial activity where as IVa and IVh showed better antifungal activity. The results were in agreement with the experimental observations.

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